SOME METHODS OF TREATING NERVE TISSUES.

WILLIAM C. KRAUSS, M. D., Buffalo, New York.

The aims to be sought after in the study of microscopy are not alone those looking to the perfection of the instrument nor those which bear upon the selection of the specimen. The preparation of this specimen, and more especially the method of staining, is as important as the specimen itself. American microscopists are more intent upon the former ; the European microscopists pay more attention to the latter. Careful study and experimentation with the different dyes have led to the compounding of stains which have enabled the microscope to reveal many of the mysteries of histology and pathology. The introduction of the aniline dyes and their successful employment by Koch lad to the discovery of the bacillus of tuberculosis, although the existence of this organism had been prophesied by writers years before its discovery. Golgi's silver-nitrate method has advanced our knowledge of the ganglion cells of the cortex of the brain, and perhaps at some day the mystery of their poles may be revealed through some simple method of staining. The importance, therefore, of the methods of staining is not to be overlooked or undervalued in the microscopical examination of tissues, and their successful employment may lead to the discovery of new facts and data of inestimable value in advancing the present status of our science.

Among some of the recent methods employed in neuro-histology and neuro-pathology, perhaps none are so important and satisfactory as the Weigert method and the Pal modification of this method. Both methods are restricted to the examination of nerve tissues, more especially of the central nervous system, where the gradation between white and gray matter is distinct and prominent.

Both methods require hardening in Müller's fluid, or simply in a saturated solution of potassium bichromate. A recent writer in Neurologisches Centralblatt, Dr. Minor, of Moscow, finds that if sections of the brain and cord are subjected to the action of the positive pole in the bichromate solution, hardening will take place

in 3, 4, or 5 days. After dehydrating and decolorizing in alcohol for some days, the preparations are ready for the imbedding mass. I have always preferred celloidin for imbedding nerve tissues and find that it is an excellent agent. It is prepared by allowing several sheets of celloidin to dissolve in equal parts of sulphuric ether and 99 per cent. alcohol. The preparations to be imbedded are placed in 99 per cent. alcohol for 24 hours, transferred to equal parts of 99 per cent. alcohol and sulphuric ether for another 24 hours, then placed for 24 hours in the celloidin solution, fastened upon corks, and are then ready for cutting.

The sections being more or less delicate and very friable, it is necessary to protect them during their passage through the various stages of the process. For this purpose collodion, photoxylin, or dextrin may be used, the *modus operandi* being as follows: Allow some of the mass to flow over a glass slide, so that a thin film remains; dry; then transfer the sections onto this prepared slide, and pour some of the mass over the sections, allowing all superfluous quantities to drip off. After a few moments the slide may be moistened in alcohol, when the sections imbedded in collodion can be removed and handled with impunity.

The Weigert method, first described by Professor Weigert, of Frankfort-a-Main, in "Fort Schritte d. Medicin," 1884, p. 190, and 1885, p. 236; also Zeitschrift f. Wissenschaftliche Microscopie, 1885, p. 490, and 1886, p. 480, requires the sections to be placed in an aqueous-saturated solution of cuprum acetate diluted with an equal amount of water for 24 hours, in a brood oven, or 48 hours in the open air. They are then washed in 60 per cent. alcohol a few hours and placed in the Weigert staining fluid:

0.75 — 1.0	parts	hæmatoxylon.
90	"	water.
10	"	alcohol.
1	"	lithium carbonate.

At the end of 24 hours they may be removed, washed in water, and are then ready for the differentiating bath:

Borax, - - - - - -	2	parts.
Ferrocyanide of potash,	2.5	"
Distilled water, - -	200	"

Some experience and great care are requisite in differentiating the sections, but with patience and judgment they can be developed

with as much precision as the photographer displays in bringing out the light and shadows of a dry plate. The sections should be placed in the bath singly, so that each one may be removed as soon as it is differentiated. The length of time required is variable, depending upon the thickness of the sections. The general outlines of the white and gray matter should be known beforehand, and if pathological the seat of the lesion, so that as soon as the contour of the different regions becomes distinct the sections may be immediately removed from the bath. Overdevelopment destroys the sections, and hence as soon as they are removed from the bath they must be thoroughly washed in water for 12, 18, or 24 hours to arrest all further development. They are then dehydrated in strong alcohol, cleared in Weigert's clearing mixture—

Xylol - - 3 parts;
Carbolic acid, 1 "
Sulphate of copper, enough to cover the bottom of the bottle,

and mounted in balsam.

The gray matter, connective tissue, and vascular walls are stained light brown; the ganglion cells, dark brown, while the white matter takes on a blackish-blue or purplish-blue tint.

The Pal Method.

This method, which is merely a modification of the Weigert method, was described by Professor Pal, of Vienna, in Wiener Medizinische, Jahrbücher, 1887, p. 589. The preliminary preparation of the sections is the same as with the Weigert method, but with care and dexterity the imbedding in collodion may be dispensed with, as they are not rendered so friable and brittle. They are immediately placed in Weigert's staining fluid, to which has previously been added 3 to 5 drops of a saturated solution of lithium carbonate for every 10 c. cm. of the stain used. After 5 or 6 hours they may be removed and carefully washed in water to which some of the carbonate of lithium solution has been added. They are now placed for 10 or 15 seconds in a ¼ per cent. solution of the permanganate of potash, rinsed in 30 per cent. alcohol, and placed in the differentiating bath.

Oxalic acid, - - 1 part.
Sulphite of soda, 1 "
Distilled water, 200 "

The same hints regarding the differentiation of the sections as given under the Weigert method are applicable in this method. Should the sections be very slow in developing, they may be replaced in the permanganate of potash solution and the process continued as before. After they have been sufficiently differentiated they should be carefully washed in water for 24 hours, dehydrated in strong alcohol, cleared in Weigert's clearing mixture, and mounted in balsam.

The medullary nerve fibers are stained light blue, the neuroglia, connective tissue, and vessel walls are rendered white or yellowish white, while the ganglion cells become transparent. To stain these cells, after the development has been arrested by the water bath, the sections should be placed in a picrocarmine stain for a short time and the process completed in the ordinary way. To stain the nuclei, the borax-carmine method must be employed as a double stain. These preparations, especially after accepting the double stain, are very pleasing to the eye and more beautiful than the Weigert specimens. I have also found that they retain their stain much better than Weigert's. A serial section made over two years ago is in as good condition as ever.

The advantages which these two methods offer must be apparent to the most astute observer. To possess a method or methods which will sharply and clearly define for us the limits and boundaries of the white and gray matter of the brain and cord is a desideratum. The differentiation between the two renders the study of pathological lesions very facile, and to follow these changes cephalad or caudad becomes a very easy matter. More especially in tracing separate bundles, or even individual nerve fibers, have these methods shown their superiority over all others. The Weigert method surpasses the Pal in this respect, being more reliable and trustworthy, and yet with the latter I have traced individual fibers in the pons and medulla with surprising accuracy. Another use to which these specimens may be put is in the study of the changes which the white and gray matter undergo in the transition between cord, medulla, pons, and crura. With the aid of a magic lantern the topography of these regions can be intelligibly demonstrated to a large class, whereas formerly these parts were almost totally ignored on account of their complex and complicated structure.

For the study of angio-pathological and ganglionic changes I prefer the carmine stains to the Pal and Weigert.